

## SEX DETERMINANT OF HUMAN HAIR WITH DNA ANALYSIS METHOD USING POLYMERASE CHAIN REACTION (PCR)

Ahmad Yudianto, Nabil A Bahasan

Department of Forensic Medicine & Medicolegal

Faculty of Medicine Airlangga University

### ABSTRAK

*Identifikasi korban maupun tersangka pada kasus-kasus kriminalitas semakin meningkat kuantitas maupun kualitasnya. Faktor lingkungan mempengaruhi DNA sehingga mengalami degradasi yang bisa cepat atau lambat, bergantung faktor yang mempengaruhi dan waktu terjadinya paparan. Kerusakan DNA dibagi menjadi 2 tipe: dari dalam, misal akibat reactive oxygen species (ROS), dan dari luar, seperti suhu dan kelembaban. Bukti di TKP berperan penting dalam identifikasi. Rambut dapat digunakan dalam penentuan ras, jenis kelamin, golongan darah. Namun sampai kini pemeriksaan melalui rambut sebagai bahan alternatif identifikasi forensik belum banyak diketahui. Penelitian ini bertujuan mengetahui pemeriksaan rambut sebagai bahan alternatif identifikasi forensik dengan DNA Profiling. Hasil penelitian ini, kadar DNA sampel penelitian yakni 20 dan 35 ul/ml. Pada visualisasi elektroforesis menggunakan polyacrylamide agarose composited gel berupa pita ditentukan apakah yang muncul 212 bp untuk amelogenin X dan 380 bp untuk amelogenin Y, dengan menarik garis pita dari sampel ke arah marker 100 bp. Pada sampel nomor 1 terdapat 2 band yakni pada 212 bp dan 380 bp maka sampel nomor 1 merupakan jenis kelamin laki-laki (XY) sedangkan pada sampel nomor 2 hanya satu band yakni pada 212 bp sehingga merupakan jenis kelamin wanita (XX).*

### ABSTRACT

*The identification of victims and suspects in criminal cases is increasing the quantity or quality. Environmental factors that affect DNA degradation that can be fast or slow, depending on factors that influence the occurrence and timing of exposure. DNA damage is divided into two types: from within, for example due to reactive oxygen species (ROS), and from outside, such as temperature and humidity. Evidence at the scene plays an important role in identification. Hair can be used in the determination of race, sex, blood type. But until now the examination through the hair as forensic identification of alternatives, not much is known. This study aims to find out the examination of hair as an alternative material with the identification of forensic DNA profiling. The results of this study, levels of DNA sample that is 20 and 35 ul/ml. On the visualization of agarose electrophoresis using polyacrylamide gel composited be determined whether the bands that appear 212 bp to 380 bp amelogenin X and Y for amelogenin, by drawing a line in the direction of the sample ribbon marker 100 bp. At sample number 1 there are two bands which is at 212 bp and 380 bp in the sample number 1 is the male sex (XY) while in sample number 2 was only one band at 212 bp so that the female gender (XX).*

**Keywords:** Sex determinant, Amelogenin, PCR

**Correspondence:** Ahmad Yudianto, Department of Forensic Medicine & Medicolegal, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Jl. Prof dr Moestopo 6-8, Surabaya 60131

### INTRODUCTION

In the case of crimes with physical violence such as murder, assault, rape and so forth, are often found blood, semen, saliva, urine, hair and other body tissues at the crime scene. Blood, semen, saliva, urine and even perspiration can be found in the form of liquid or dry form of patches that have been attached to an object in the scene is. Such materials may come from the victim or the suspect (criminal) or of both, so it can be used as materials inspection to reveal the occurrence of crime scientifically. Hair was among the evidence found at the scene. In cases of sexual crimes hair examination plays an important role. In the study of hair examination can reveal whether the hair came from the body of the victim or the suspect or perpetrator of crime or even

instead of a human hair. To date the examination through the hair as forensic identification of alternatives, not much is known. No studies of sex determination in the hair with the use of methods of DNA analysis amelogenin gene.

Hair in humans there are three kinds of hair, either at the head or sex, is part of the body that can provide a wealth of information for the interests of justice. One is the identification checks. In the process of identification, although not able to provide personal identity, but at least your hair can give information about age, gender, race and blood type. The value of hair evidence is high when the case is not found any evidence or when evidence is found to have been damaged. In the determination of sex through the hair can be done by

analysis of DNA profiling. Important proteins that play a role in sex determination is amelogenin. Amelogenin gene which encodes a protein located on chromosome X and Y in humans. The focus of this study is to reveal the hair follicle epithelial cells in which there can determine the sex by analysis of DNA by PCR method.

## MATERIALS AND METHODS

The design of this study was an observational laboratory to determine the sex determinant through the hair cells by the method of DNA Profiling. The material in this study was derived from the DNA of hair strands were 5 T4 bodies. The study was conducted at the Human Genetic Study Group, Institute of Tropical Diseases (ITD). Studies using PCR Cycle (9700, Applied Biosystem), Spectrophotometer, electrophoresis, Whirlimixer, Centrifuge, Ependorf and micropipette tips White, Yellow & Blue, Transsonic 310 (Elma), Spinator (Millipore), Tubes ependorf 0.5 cc, 1.5 cc and 2 cc, microwave, and electric scales. For data collection procedures performed on hair DNAzol DNA extraction reagent (Invitrogen), measurement of DNA content and purity by spectrophotometer. Then performed PCR amplification and composited polyacrylamid agarose gel electrophoresis stained with silver staining.

## RESULTS

Results of DNA levels after isolation of DNA from hair and purity of DNA prior to PCR amplification (Polymerase Chain Reaction) as follows:

Table 1. Levels and purity of DNA on the hair

Samples	Quality ( $\lambda$ 260/ $\lambda$ 280)	Level ( $\mu$ g/ml)
1	1.21	35
2	1.1	20

Levels of DNA isolation from the hair of two samples each: 35? G/ml and 20? G/ml. Levels of DNA that can still be used in the process of DNA profiling, according to Notosoehardjo (1999) requires the number or DNA content of about 20? G/ml for typing. The purity of the sample DNA isolation results: 1 - 2 (ideally 1.8), allowing for PCR amplification. In the visualization of agarose electrophoresis using polyacrylamide gel composited determined whether in the form of ribbons that appear 212 bp to 380 bp amelogenin X and Y for amelogenin, draw a line from the sample towards ribbon marker 100 bp. In sample 1 there are two bands namely 212 bp and 380 bp, sample 1 XY sex while on the

sample number 2 was only 1 band at 212 bp that is so is the sex of XX.

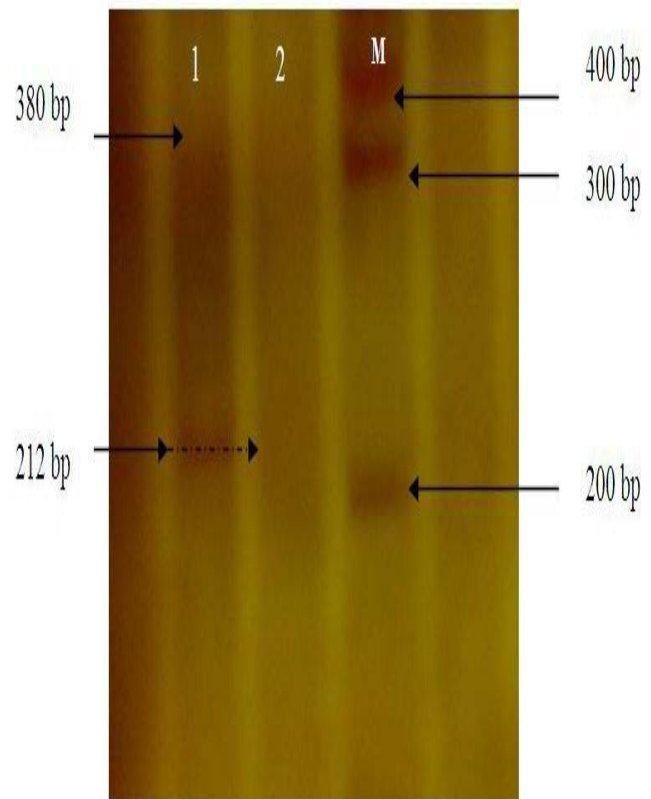


Figure 1. Visualization of electrophoresis results

M : Marker ladder 100 bp

1 : Sample 1 showed 2 bands, 212 bp (X) and 380 bp (Y)

2 : Sample 2 showed 1 band, 212 bp (X)

## DISCUSSION

Forensic Medicine is part of the Medical Sciences, its role in handling victims of mass disasters ever increasing number and scale, as well as upholding justice and the disclosure of various criminal cases in the homeland (Atmaja 2005). As well as the identification of victims of the earthquake and tsunami disaster in Aceh, the end of 2005, the identification of victims of Bali bomb blast 1, which claimed 182 people died through the identification of Dr.. Azhari adding a long row of the role of Forensic Medicine in the homeland.

Not to mention the identification of victims and suspects in cases of crime such as murder, rape with homicide are increasingly also increase the quantity and quality, increasingly confirmed the existence of forensic

medicine, as a branch of medical science that deserves to be taken into account its role. This is certainly not independent of the increasingly complex task for the forensic identification of current and in the days to come, as in the case of crimes which are accompanied by efforts to remove traces of the suspect, ie by shredding the victim into several sections, or by burning victims who bring degrees of damage due to the great body of the victim (Notosoehardjo 1999). This condition is certainly not uncommon to make forensic identification can not be enforced through the items or something that is personal, such as ID documents, watches, rings, or known by the term associative identification.

One of the most important identification is the sex determination (sex determinant). Sexes has several different meanings, namely chromosomal, chromatin, gonadal, hormonal, somatic, and psychic metrical. The sex is determined chromosomally sexual chromosomes. Chromatinal gender is determined by the X sex chromatin (Barr body). The sex chromosomal and chromatin called gender genetics. Gonadal gender is determined by the testes in men and in women who ovaria began to take shape beginning in the seventh intrauterine. The sex hormonal or metabolism is determined by the quality and quantity of sexual hormones. Both gonads produce both androgens and estrogens, but far more androgens are produced by the testes and much more estrogen by ovaria. Androgens are also produced by glands kidneys child so that the levels of androgens and estrogens in the blood serum can lead to errors in determining the sex hormones. While sex is determined by somatic genital development and external genitalia in the penis and scrotum in males and vagina in women, gender is generally apparent in the form of somatic, height, volume, body proportions, pilositi, skull shape, pelvic shape and limb shape. Metrical gender (birth certificates, social) is determined at birth based on the external genitals. Psychological gender is the feeling that sex is determined in ontogenetic development and education.

In its development, sex determination is done by biomolecular method, namely through PCR (Polymerase Chain Reaction). One of the PCR techniques for identification of sex is by amplification of the XY homologous gene amelogenin (Butler 2003). Amelogenin is a major constituent of the enamel matrix protein formation. There are two amelogenin gene, one on the X chromosome and the other on the Y chromosome Map of the human amelogenin gene on the p22 region on chromosome X and the Y chromosome genes have been mapped. Typing amelogenin gene can be used for sex determination in biological samples.

Amelogenin is relatively easy to use for DNA amplification. By using a single primer pair that covers most of the first intron, will produce a PCR product of X and Y homologues, which is then described by a gel polyacrylamide electrophoresis. PCR method proved to have widespread uses for forensics because of its simplicity, sensitivity and reliability and also superior because it can theoretically detect the sex of one cell and much faster.

In this research, using hair root (hair roof) as an ingredient of personal identification, the results of this study found that the hair can be used as alternatives in personal identification. Histologically, the structure of hair, hair epithelial cells have a nucleus at its root area (roof). Levels of DNA from the hair of the results of this research were still enough in the examination of DNA typing. Levels of DNA are an important factor in forensic DNA examination. This is due to levels of influence on the success of STR DNA genotyping on DNA samples. According to Bergen, believes the potential decrease of 1 ng to the decrease of detection capability up to 95% STR.

With regard to the levels of DNA, about the minimum levels that can be used in forensic DNA analysis is still no definite measure. According to Michelle, minimal levels of DNA in DNA typing is required ranged from 0.1 to 50 ng. Meanwhile, minimum required levels is ranging from 20-50 ng. In the butler said that the inspection is good with the STR method of DNA with a minimum concentration of between 0.5 to 2.5 ng. Besides depending on the DNA content of the examination materials, the examination of DNA by PCR is also required that sufficient DNA quality. Adequacy of the quality of the DNA that the DNA used in the degraded condition to a minimum. If the DNA in degraded conditions resulting in severe primary or anealing can not stick to the target DNA to be duplicated. So that good quality DNA to be a fundamental prerequisite for the success of the PCR reaction as a whole.

In this research, the primary amelogenin still capable to amplify so the visualization results are still visible image of the bands, ie, in sample 1 of 2 bands (212 bp and 380 bp). The results of DNA bands from the hair is still not so perfect or vague, this is because the levels of DNA produced is still quite low. Or even because of DNA degradation process. As is known, moisture, exposure to high temperature and disorder or a bacterial or fungal contamination and therefore contributes to the condition of the DNA. According to Hofreiter (2001) basically DNA damage or degraded DNA can be divided into two main types, namely: first the damage from inside or endogenous damage, as well as damage

caused by reactive oxygen species (ROS) due to the process of oxidative phosphorylation and the DNA damage caused by factors external or exogenous damage, such as ultraviolet radiation from the sun or radiation from other sources, such as x-rays and gamma rays and thermal disruption

In this study, samples of hair taken from the body of T4 (not permanent residence) in accordance with statutory provisions in force in Indonesia, namely the Criminal Procedure Code (the book of criminal procedure) that when the evidence in this case the corpse if the 2 x 24 hours is not which recognizes the existence of family or relatives is not known then the corpses was government owned and treated in accordance with existing regulations. So here, the hair was drawn from bodies that had died more than 2 days. Factors affecting DNA damage can begin to take place. These factors ie endogenous factors, or better known as post mortem damage. The occurrence of DNA damage is concurrent with autolysis, several hours or several days after death (Hofreiter et al. 2001). DNA damage will get worse along with increasing 'age' of individual death, DNA is often expressed in a broader fragmented. Oxydative DNA damage causes loss of nitrogenous bases. While the hydrolytic damage caused depurination and depyrimidation of DNA bases, which ultimately resulted in the shortening of DNA fragments, since the occurrence of bond cleavage of phosphate or phosphate-link or by other terms known as DNA fragmentation (Butler et al. 2003)

## CONCLUSION

From the results of this study was that the isolation of DNA from the hair can still be an alternative material in the forensic identification of sex determination. Generally, isolation of DNA from hair provides low-level or quantity of DNA or even less, but DNA band is still visible on the results of electrophoresis visualization. Factors influencing the DNA content of the DNA damage are caused by endogenous and exogenous factors.

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